Environmental Health Perspectives
Vol. 40, pp. 197-205, 1981

Metal-Induced Infidelity of DNA Synthesis

by Richard A. Zakour,* Thomas A. Kunkel,* and Lawrence A. Loeb*

In this paper, we consider the effects of metal ions on the accuracy of catalysis by DNA polymerases. Certain activating and nonactivating metal ions have been shown to diminish the fidelity of DNA synthesis *in vitro* with a variety of DNA polymerases. There is a significant correlation between the metals that decrease fidelity and those that have been reported to be mutagenic and carcinogenic. Thus, metal carcinogens are no exception to the general postulate that carcinogens can be identified by their interactions with DNA.

The Present State of Knowledge

A number of metals have been shown to be mutagens and carcinogens (*1*) and to affect the accuracy of DNA replication (*2*). *In vivo* systems are too complicated to begin to unravel the mechanisms by which metals induce mutations and the effects of metal ions on the fidelity of DNA replication. Our approach to this problem has been to examine DNA synthesis *in vitro*, to determine the effects of different metal ions on the fidelity of this process, and then to ask whether alterations in the fidelity of DNA synthesis are related to the mutagenic and carcinogenic properties of these metals. Prior to considering these studies in detail, it is instructive to consider the mechanism of DNA synthesis *in vitro* and methods for measuring fidelity of DNA synthesis.

Mechanism of DNA Polymerization

The requirements for catalysis by various DNA polymerases appear to be similar. Synthesis proceeds by a sequential addition of nucleotide monomers (deoxynucleoside monophosphates) with the concomitant release of pyrophosphates (*3*). DNA polymerases are part of a unique class of enzymes in that they primarily take direction from another molecule, a template. In cells, the template is DNA. Synthetic polydeoxynucleotides and polyribonucleotides can also serve as templates for most DNA polymerases *in vitro*. Synthesis is started on the 3′-hydroxy terminus of a primer-strand hybridized onto a template strand. The primer can be an oligonucleotide, one strand of double-stranded DNA, a hairpin loop of single-stranded DNA, or a fragment of RNA hybridized onto DNA. Thus, DNA polymerases only elongate already existing polynucleotide chains; they fail to initiate chains *de novo* as do RNA polymerases. The substrates of all known DNA polymerases are deoxynucleoside triphosphates that are complementary to the template. Based on the similar requirements for activity and a spectrum of similar kinetic parameters, it is a reasonable expectation that there is a common mechanism for catalysis by DNA polymerases from different sources (*4*).

DNA polymerases can be classified as zinc metalloenzymes (*5*) in that they contain tightly bound zinc, which is required for activity (*6*). In addition, these enzymes require an added metal for catalysis, which in cells is presumably Mg^{2+}. *In vitro*, Mg^{2+} has been shown to coordinate the enzyme with the substrate in the form of an enzyme-metal-substrate complex. Mn^{2+}, Co^{2+}, Ni^{2+}, and in certain cases, Zn^{2+}, have been shown to substitute for Mg^{2+} as activators (*7*). Analysis of *E. coli* DNA polymerase I-Mn^{2+}-substrate complexes indicates that in the absence of template, the

* The Joseph Gottstein Memorial Cancer Research Laboratory, Department of Pathology, University of Washington, Seattle, Washington 98195

enzyme alters the conformation of the deoxynucleoside triphosphate substrate to that which it would occupy in double helical DNA (*8*). The function of the metal activator in the DNA polymerase reaction *per se* has recently been extensively reviewed (*9, 10*).

Fidelity of DNA Synthesis

On the basis of spontaneous mutation rates in procaryotic and eucaryotic cells, stable misincorporation of a base during DNA replication is estimated to occur with a frequency of 10^{-8} to 10^{-11} per base-pair synthesized (*11*). This accuracy appears to be achieved by a multistep process (*12*). The differences between correct and incorrect Watson-Crick base-pairings involve only one or two hydrogen bonds. This difference in free energy, ΔG has been estimated to account for an error rate of approximately 10^{-2} (*13*). DNA polymerases also participate in base-selection, reducing the error rate to values approaching 10^{-5} (*4*). Presumably, other cellular mechanisms increase accuracy during or immediately after DNA replication. At present, studies on the effects of metals on fidelity *in vitro* are limited to analysis with purified DNA polymerases.

Assays of Fidelity with Polynucleotide Templates

Until very recently, all assays of the fidelity of DNA synthesis *in vitro* measured the ability of DNA polymerases to copy homopolymer or alternating copolymer templates. These templates contained only one or two nucleotides and the mismatched nucleotide was identified simply as one not complementary to the template nucleotides. Using this assay, one can observe the effects of both activating and non-activating metals on the fidelity of DNA synthesis. The template that we have chosen for critical measurements of fidelity is poly[d(A-T)], a synthetic polynucleotide consisting of deoxythymidine and deoxyadenosine monophosphates. Poly[d(A-T)] can be synthesized to contain less than 1 in 2×10^6 mistakes by using a *de novo* reaction with *E. coli* DNA polymerase I (*14*). Copied correctly, only dAMP and dTMP should be incorporated into the newly synthesized product. By using [α-^{32}P]-dTTP, unlabeled dATP, and [^{3}H]-dGTP or [^{3}H]-dCTP, one can simultaneously measure the incorporation of complementary and non-complementary nucleotides (*15*). The incorporation of either dCTP or dGTP would represent errors. The frequency of misincorporation is obtained from the ratio of [^{3}H] to [^{32}P] in the acid-insoluble product. Control experiments are required to show that the [^{3}H] label in the reaction product is in the noncomplementary nucleotides and not in any radioactive contaminants. Also, it must be demonstrated that the noncomplementary nucleotides are covalently incorporated in phosphodiester linkage. Using nearest-neighbor analysis, one can determine the distribution of the noncomplementary nucleotides. Measurements of the frequency of misincorporation by DNA when copying polynucleotide templates have been summarized in a recent review (*12*). In general, they vary from 10^{-3} for DNA polymerases from RNA tumor viruses to 10^{-5} for procaryotic DNA polymerases. It should be noted that the error rates of the procaryotic DNA polymerases, those with a $3' \rightarrow 5'$ exonuclease, are similar to those of the eucaryotic DNA polymerases, enzymes that do not have an accompanying exonuclease (*16*). Thus, the exonuclease in procaryotic DNA polymerases is not necessarily the major determinant of fidelity.

Metals Activators and Fidelity

The activating metal for DNA polymerase *in vivo* is Mg^{2+}. *In vitro*, DNA polymerases from animal (*16*), viral (*17*), and bacterial sources can also use Mn^{2+}, Co^{2+}, or Ni^{2+} as the activating metal. With AMV DNA polymerase and an activated DNA template, the maximal rates of nucleotide incorporation with Mn^{2+}, Co^{2+}, and Ni^{2+} were 65%, 25%, and 7%, respectively, of that achieved with Mg^{2+} (*18*). Minimal activity has also been reported with Zn^{2+}. The effects of Mg^{2+} and Mn^{2+} concentrations on the incorporation of complementary and noncomplementary nucleotides with poly [d(A-T)] as a template are illustrated in Fig. 1. At activating concentrations of Mg^{2+} (2*mM*), human placenta DNA polymerase-β incorporates one molecule of dGTP for every 40,000 molecules of dTTP and dATP polymerized (*17*). This error rate is invariant with respect to Mg^{2+} concentration. At the optimal activating concentration of Mn^{2+} (0.1*mM*), the error rate was 1 in 15,000. At greater than activating concentrations of Mn^{2+}, there was a progressive decrease in the incorporation of the complementary nucleotide but not of the noncomplementary nucleotide, thus yielding a further increase in the frequency of misincorporation. At concentrations as great as 2*mM*, the error rate approached 1 in 3600, and nearest-neighbor analysis indicated that each misincorporation occurred as a single-base substitution. A similar enhancement in the error rate is observed with both activating and inhibiting concentrations of Co^{2+}. The decreased fidelity with increased Mn^{2+} concentration has been observed

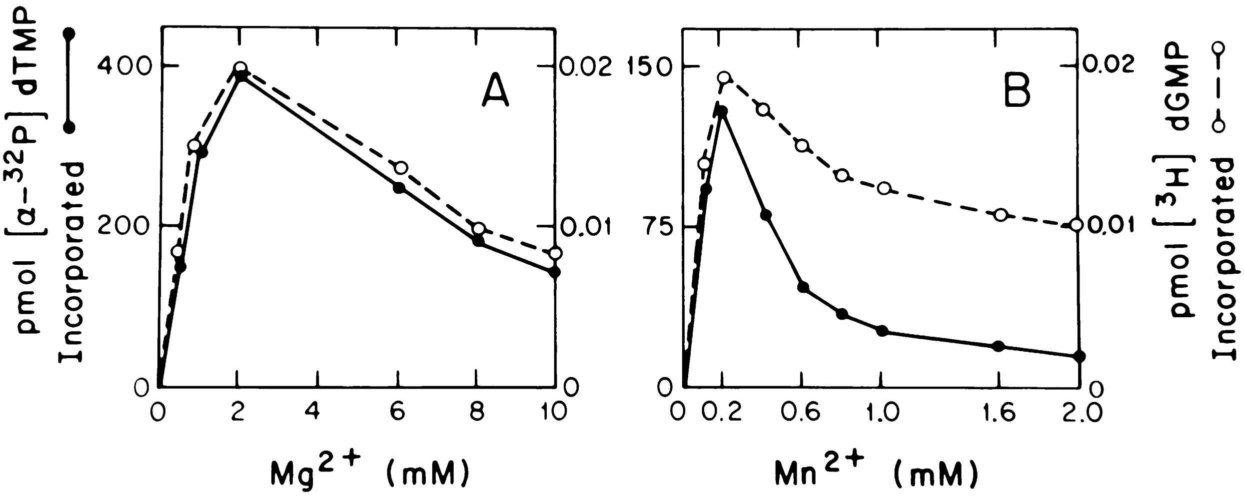

FIGURE 1. Effect of metal activators on the fidelity of human placenta DNA polymerase-β with poly [d(A-T)] as a template (*17*).

with all templates and noncomplementary nucleotides tested. An absolute increase in the rate of incorporation of the noncomplementary nucleotide at high Mn^{2+} concentration can be demonstrated by simply using more DNA polymerase in the assay or prolonging the time of incubation.

The enhancement of misincorporation with an alternate metal activator appears to be a characteristic finding with DNA polymerases (Table 1). Substitution of Mn^{2+} for Mg^{2+} results in an increase in misincorporation by *E. coli* DNA polymerase I (*7*), T_4 DNA polymerase (*19*), DNA polymerase-α (*17, 20*) DNA polymerase-β (*17*), and avian myeloblastosis virus (AMV) DNA polymerase (*15, 21*). The fact that Mn^{2+} and Co^{2+} alter the fidelity of the DNA polymerases that do not have an associated exonuclease (AMV, DNA polymerases-α and -β) indicates that for these DNA polymerases the metal ions do not promote misincorporation by inhibiting an error correcting exonucleolytic activity. Ni^{2+} can also substitute for Mg^{2+} as a metal activator. However, the amount of synthesis achieved with Ni^{2+} as the metal activator has not been sufficient to accurately measure the changes in the fidelity of DNA synthesis with any DNA polymerase except AMV DNA polymerase, in which case Ni^{2+} promotes misincorporation (*18*).

In order to relate the measurements with alternate metal activators to a situation that would be expected to occur in cells, the effects of these activators on Mg^{2+}-activated DNA synthesis have been investigated. Co^{2+}, Mn^{2+}, and Ni^{2+} have been shown to enhance misincorporation by DNA polymerases in the presence of activating amounts of Mg^{2+} (*17, 21, 22*). Thus, these metal activators could alter the fidelity of DNA polymerases in cells.

Nonactivating Metal Ions and Fidelity

Beryllium, a known carcinogen, has been shown to decrease the fidelity of catalysis with *M. luteus* DNA polymerase (*23*) and AMV DNA polymerase (*24*). Be^{2+} is unable to substitute for Mg^{2+} as a metal activator. However, as a nonactivating cation,

Table 1. Error rates with different metal activators.[a]

DNA polymerase	Mg^{2+} (5*mM*)	Mn^{2+} (0.1 *mM*)	Mn^{2+} (2 *mM*)	Co^{2+} (0.4 *mM*)	Co^{2+} (5 *mM*)
AMV	1/1,680	1/760	1/500	1/1,100	1/200
E. coli I	1/20,000	1/10,000	1/1,000	1/7,500	1/7,000
Human placenta-α	1/6,000	1/1,900	1/300	1/1,300	1/450
Human placenta-β	1/20,000	1/9,000	1/2,000	1/5,000	1/1,300

[a]All assays were carried out with poly [d(A-T)] as a template and dATP, [α^{32}P]dTTP and [^{3}H]dGTP at 25 μM (*12*).

Be^{2+} alters the fidelity of DNA synthesis in the presence of Mg^{2+}. Preincubation of the enzyme, but not the template, primer or substrates with high concentrations of Be^{2+} resulted in an increased error rate (*24*). This finding suggests that Be^{2+} can interact with some noncatalytic site on DNA polymerase and thereby alter the fidelity of DNA synthesis. Be^{2+} has also been shown to alter the fidelity of DNA polymerase-α from human fibroblasts (*25*), DNA polymerases-α and -β from human placenta and *E. coli* DNA polymerase I (*17*). Since the eucaryotic and viral DNA polymerases lack an exonuclease, these results mitigate against the hypothesis of Luke et al. (*23*) that Be^{2+} interacts with the exonucleolytic site on procaryotic DNA polymerases.

Screening for Metals that Alter Fidelity

To date, over 40 metal compounds have been tested in graded concentrations for their effects on the fidelity of DNA synthesis. The method of analysis and the results are summarized in Figure 2. In the initial study, 22 of these metal salts were tested by using a triple-blind protocol in which the assays, computations, and designation of each unknown compound with respect to fidelity were carried out independently (*2*). Compounds which increased infidelity by greater than 30% at two or more concentrations were scored as positive. Metals were designated as carcinogens or mutagens by an evaluation of the literature prior to assessment of their effects on fidelity. An enhancement in the infidelity of DNA synthesis was observed with all of the known mutagens and/or carcinogens tested at that time (Ag, Be, Cd, Co, Cr, Mn, Ni, Pb). The evidence in the literature on the mutagenicity or carcinogenicity of three of the metal ions was considered equivocal. Of these, Cu^{2+} increased misincorporation; Fe^{2+} and Zn^{2+} did not alter fidelity. All other metal salts that were tested were considered to be neither carcinogenic nor mutagenic, and they did not increase misincorporation. Only a few of the metal salts that did not alter fidelity are listed in Figure 2.

With only a few exceptions, these results have been confirmed by Miyaki et al. (*22*) and Sirover et al. (*7*), using *E. coli* DNA polymerase I, and by Seal et al., using DNA polymerases-α and -β from human placenta (*17*). Most recently, we have observed that neither arsenic (AsO_4, As_2O) nor selenium (SeO_2) diminish fidelity with *E. coli* DNA polymerase I (*26*). Furthermore, Se, which has been reported to have an anticarcinogenic effect (*27*), does not reduce the mutagenic effect of Mn in titration experiments containing varying amounts

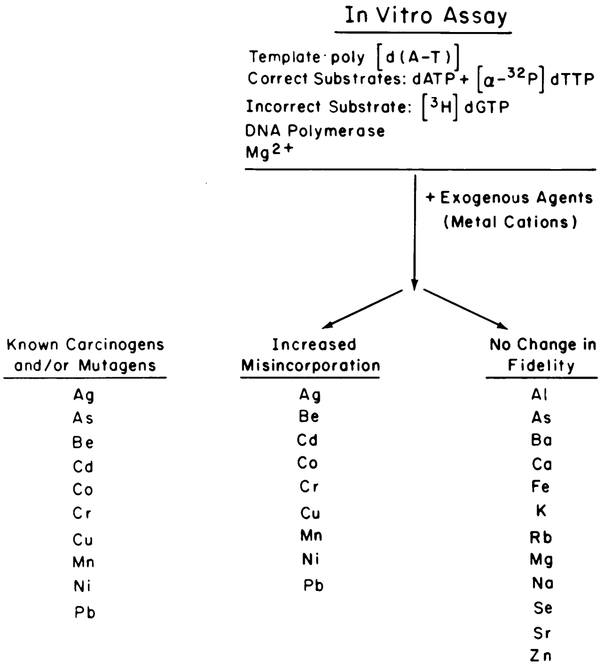

FIGURE 2. Screening for metal ions that alter fidelity. The data are compiled from the literature (*2, 26, 32*).

of these two metal ions. We have also examined the effect of different ionic species of chromium and have found that both Cr (III) and Cr (VI) alter the fidelity of *E. coli* DNA polymerase I (*26*). Chain initiation by RNA polymerases can be stimulated to Pb^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+} and Mn^{2+}, whereas Zn^{2+}, Mg^{2+}, Li^{+}, Na^{+} and K^{+} are inhibitory (*28*). The similarity between the effects caused by particular metal ions on fidelity with DNA polymerases and on chain initiation with RNA polymerase could point to metal interactions with the DNA template as the common underlying mechanism for these two phenomena.

Fidelity of DNA Synthesis with Natural DNA Templates

All of the aforementioned studies on the effects of metal ions on the fidelity of DNA synthesis depended on measuring the incorporation of noncomplementary nucleotides by using synthetic polynucleotide templates of limited composition. It has been assumed that the results with such a model system are similar to those that would be obtained copying natural DNA containing all four bases. It is known, however, that slippage of the primer relative to the template can occur when primed templates of a repeating nucleotide sequence are copied. Thus, metal-mediated changes in the fidelity of DNA synthesis could result from such slippage of the primer on the template, an event that presumably does not occur during copying of natural DNA templates. Also unique to homopolymers or repeating heteropolymers is the fact that a single noncomplementary nucleotide can occupy a looped-out structure without changing the reading frame of subsequent codons. Thus, metals could enhance misincorporation by increasing the frequency of such looped-out structures. To circumvent these limitations, a system has been recently developed (*29*) to monitor the fidelity of *in vitro* DNA synthesis using a natural DNA template, DNA from the bacteriophage φX174 carrying a suppressible nonsense mutation, amber 3 (*am3*) (Fig. 3). Certain nucleotide substitutions within the *am3* locus that occur during *in vitro* replications of this DNA will cause a reversion to the wild type phenotype. Such reversions are detected by the bioassay method diagrammed in Figure 3. Thus, measurement of the reversion frequency of the progeny phage indicates the accuracy with which the DNA in the region of this mutation was copied.

This assay system has been used with AMV DNA polymerase (*30*) and *E. coli* DNA polymerase I (*31*). With homogeneous AMV DNA polymerase Mg^{2+}, and equal concentration of nucleotides, the *in vitro* mutation rate is approximately 1 in 1000. With *E. coli* DNA polymerase I, variations in the divalent metal ion activator used in the copying reaction markedly affects the reversion frequency of copied φX174 *am3* DNA. Thus, the calculated error rate observed with 5*mM* Mg^{2+} can be increased severalfold by the substitution of Mn^{2+} or Co^{2+} for Mg^{2+}. The error rate can also be increased by copying in the presence of inhibiting concentrations of Mg^{2+} or by the presence of Cr^{3+} or Cr^{6+} in Mg^{2+}-activated reactions (*32*). These limited studies suggest the metal mutagens and carcinogens also diminish accuracy with natural DNA templates. By determining the sequence of the DNA synthesized in the presence of mutagenic metals using the φX174 DNA template, it should be possible to define the specificity of interactions of these metals with the template nucleotides.

Mechanism of Genetic Miscoding by Metals

The exact mechanism by which certain divalent metal ions decrease the fidelity of DNA synthesis *in vitro* is not known. On the basis of the available data, three alternatives can be unambiguously eliminated, while three others may still be considered viable mechanisms and will require further investigation.

The following three possibilities by which metal

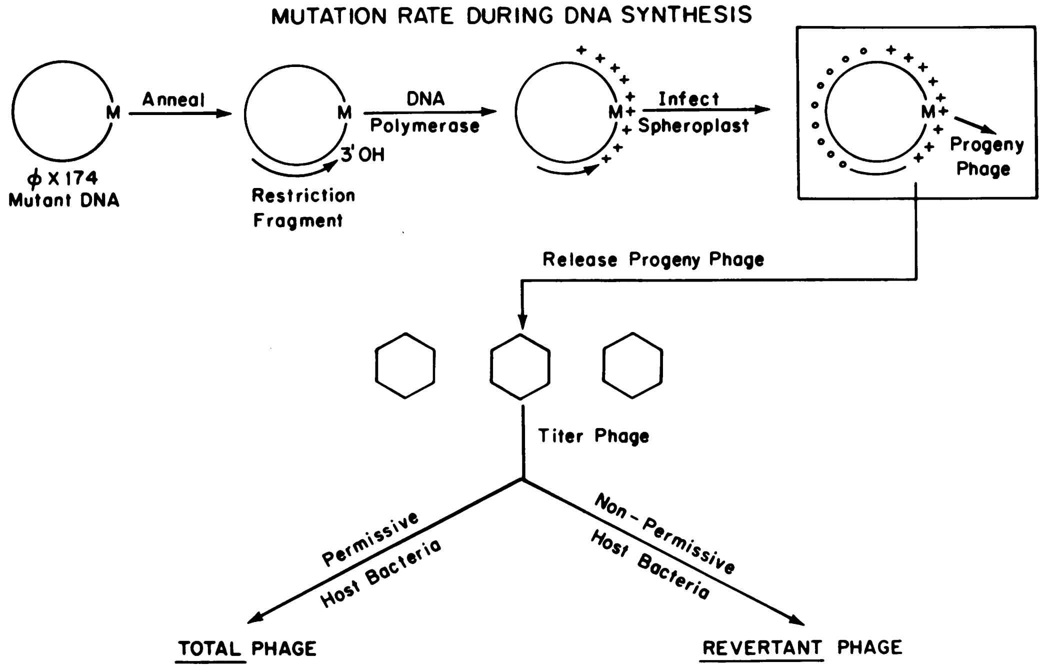

FIGURE 3. φX174 fidelity assay (*29*, *31*).

ions decrease the fidelity of *in vitro* DNA synthesis are no longer tenable mechanisms.

Precipitation of Noncomplementary Nucleotides. It can be argued that the observed increase in error frequency at high metal concentration represents the selective acid precipitation of metal ion complexes containing unincorporated noncomplementary nucleotides. However, physical and enzymatic studies of the products synthesized with AMV DNA polymerase (*33*), *E. coli* DNA polymerase (*7*), and DNA polymerases-α and -β (*17*), indicate that the noncomplementary nucleotides are incorporated into a polynucleotide chain, predominantly as single-base substitutions.

Metal-Substrate Interactions. Metal-induced infidelity does not appear to result from selective interactions between particular metals and particular nucleotides. For example, it could be argued that Co^{2+} selectively interacts with the complementary nucleotide and reduces its effective concentration in the reaction mixture. However, at a high concentration of Co^{2+} (5*mM*), the incorporation of dGTP as the complementary nucleotide with a poly (C) template is markedly inhibited, whereas the incorporation of dGTP as the noncomplementary nucleotide with poly [d(A-T)] as the template is undiminished (*18*). Similar results have been obtained with Mn^{2+} using various DNA polymerases with different template combinations.

Inhibition of "Proof-reading" Exonuclease by Metal Ions. The possibility that decreases in fidelity with divalent metal ions are mediated by inhibition of 3′ → 5′ exonucleolytic activity is also unlikely. Eucaryotic DNA polymerases and DNA polymerases from RNA tumor viruses are devoid of such an activity (*16*), yet mutagenic metal ions decrease the fidelity of these enzymes. Detailed studies on the effect of Mn^{2+} on fidelity, exonucleolytic activity, and monophosphate generation have been carried out with *E. coli* DNA polymerase I. Under conditions in which Mn^{2+} diminishes fidelity, there is no diminution of the 3′ → 5′ exonucleolytic activity (*7*).

The decrease in fidelity of metal ions during *in vitro* DNA synthesis can be explained most directly by any one or more of the following types of interactions (Fig. 4).

Altered Substrate Conformation. The ability of Mn^{2+}, Co^{2+}, Ni^{2+}, and possibly Zn^{2+} to substitute for Mg^{2+} as a metal activator focuses on the possibility that the mechanism of change in fidelity by these metals occurs by a substitution at the substrate binding site on the polymerase. Using a variety of DNA polymerases, the frequency of misincorporation at activating concentrations of Mn^{2+} and Co^{2+} is 2- or 3-fold that observed with

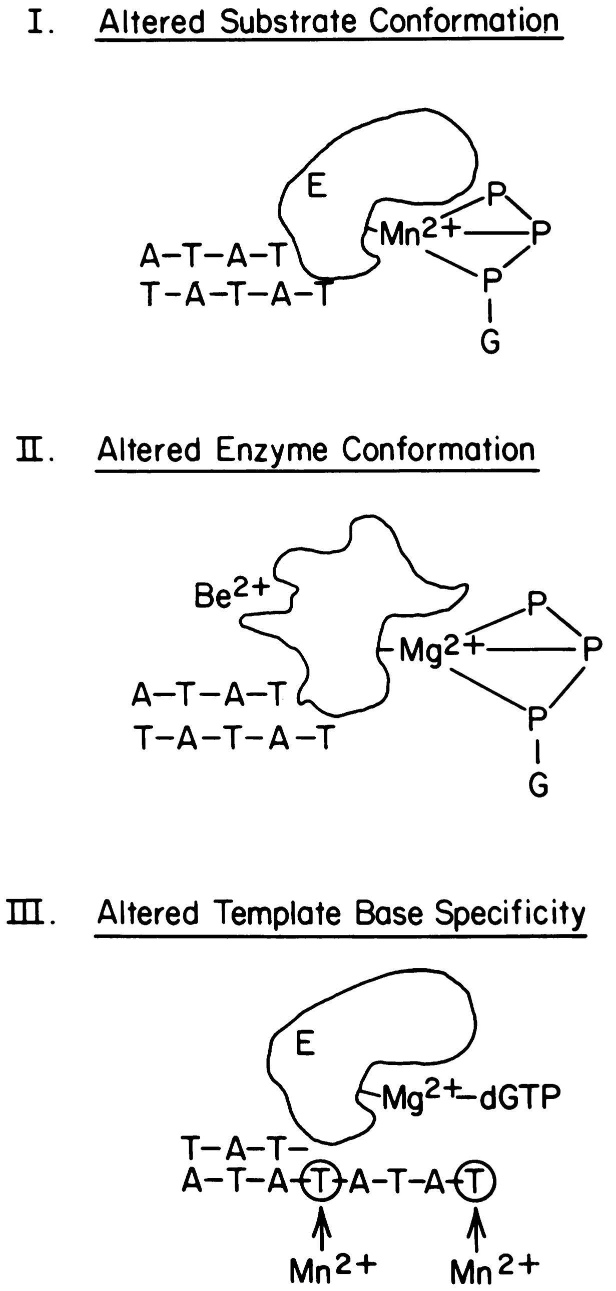

FIGURE 4. Possible mechanisms for metal-induced infidelity during DNA synthesis (see text).

Mg^{2+}. Magnetic resonance studies indicate that the interaction of the metal activator involves an enzyme-metal-substrate bridge complex involving the γ-phosphoryl group of the substrate (*35*). Studies with *E. coli* DNA polymerase I in the absence of template indicate that the bound metal changes the conformation of the substrate to that of the nucleotidyl unit in double-helical DNA. Sloan et al. (*8*) noted that this conformation could reduce the frequency of misincorporation. Thus, it could be argued that differences in conformation of the bound substrate with different metal activators might account for differences in the fidelity of DNA synthesis, particularly at activating concentrations of these cations. However, the current data are not sufficient to eliminate the possibility that differences in fidelity reflect interactions of metal ions with the template and not the enzyme even when the metal serves as an activator. Thus, the parallel incorporation of complementary and noncomplementary nucleotides at activating metal concentrations could simply indicate that polymerization is a rate-limiting event and the metal-mediated change in fidelity could be at a site other than the substrate site on the enzyme.

Altered Enzyme Conformation. The decrease in fidelity observed at inhibiting concentrations of metal activators suggests binding of metals at sites in addition to the catalytically active site. Ancillary binding sites for Mn^{2+} were detected on *E. coli* DNA polymerase I by nuclear magnetic resonance studies (*35*). The demonstrations that nonactivating metal ions alter the fidelity of other DNA polymerases is compatible with this concept. Also, evidence has been presented that Be^{2+}, a nonactivating cation, binds to AMV DNA polymerase directly and diminishes the fidelity of DNA synthesis *in vitro* (*24*). Thus, interactions of metals or metal-nucleotide complexes at distant sites could change the conformation of the polymerase so as to promote misincorporation. To date, attempts in our laboratory to generate an altered DNA polymerase with diminished fidelity by treatment with denaturing agents and heat have not been successful.

Altered Template-Base Specificity. The direct interaction of metal ions with phosphates and bases on polynucleotides have been measured by a number of physical techniques (*36*). Studies on the interaction of Mn^{2+} with activated DNA template by paramagnetic resonance (*35*) indicate 5 ± 2 very tight sites and 52 weaker sites having an invariant association constant of 68 μM. The largest decreases in fidelity with Mn^{2+} were observed at much higher concentrations (2-5*mM*). Weak Mn^{2+} binding sites on *E. coli* DNA polymerase I have been reported (*35*). However, it is also possible that very weak binding sites on polynucleotides are responsible for diminished fidelity, and these would not be observed in the magnetic resonance experiments. Eichhorn and collaborators initially observed that metal ions can cause enhanced mispairing upon renaturation of polynucleotides (*36*). Conceivably, the metal ions can directly interfere with complementary base-pairing or cause a shift in the keto-enol equilibria of the nucleotide. Recent studies by Murray and Flessel (*37*) indicate that Mn^{2+} and Cd^{2+} promote mispairing during hybridization of the synthetic templates. Moreover, the mispairing with Mn^{2+} can be demonstrated to occur at millimolar concentrations.

Public Health Implications

The results in this working paper suggest that mutagenic metal ions alter the fidelity of DNA synthesis. This has been demonstrated with purified DNA polymerases using both synthetic and natural DNA templates. We argue that in studying fidelity of DNA synthesis by DNA polymerases, one is studying mutagenesis *in vitro*. Correlations observed between alterations in fidelity *in vitro* and mutagenicity or carcinogenicity *in vivo* are in accord with the hypothesis that infidelity during DNA synthesis may cause mutations. However, we recognize that metal ions have many other effects *in vivo*. Considerable evidence will be required to document whether or not alterations in the fidelity of DNA synthesis are causally associated with mutations and malignancy. Irrespective of a defined mechanism, the correlation between alterations in fidelity and mutagenicity and/or carcinogenicity indicates the practicality of using fidelity assays as a screen for evaluating possible mutagens and carcinogens. Since these assays are carried out *in vitro* in defined homogeneous systems, it is possible to design experiments to understand how metals alter the fidelity of DNA synthesis.

With respect to metals, diminished fidelity and somatic mutations, the following hypothesis can be generated. Metal-induced mutations may occur by the interaction of metal ions with the DNA template or with the DNA polymerase. In the latter case, a normal polymerase could be exposed to an abnormal concentration of physiologically required metals, or to exogenous metals that are usually not present during cellular metabolism. Alternatively, metal ions that are normally not used for DNA replication could serve as activators for DNA polymerases that have been previously altered. In either case, an abnormal polymerase-metal combination might decrease the fidelity with which the DNA is replicated, and thus lead to the synthesis of DNA containing mutations. This newly synthesized DNA may contain certain critical errors (e.g., genes which code for altered polymerases). Furthermore, continued replication of the DNA by an altered polymerase or in the presence of mutagenic metals could also lead to an accumulation of additional errors during subsequent rounds of replication. Such critical errors and/or cascading errors caused by an accumulation of mutations may account for the progressive change in cellular properties during tumor progression.

Required Research

To date, only the initial studies have been conducted on genetic miscoding by metals. The main gap in our knowledge concerns the relationship between the studies with metals and purified polynucleotides *in vitro*, as well as those involving infidelity by DNA polymerases, to the realities of chromosomal replication in cells. The central question is whether or not the infidelity of DNA synthesis by metals is causally associated with mutagenesis and perhaps carcinogenesis. The approach to the problem requires an analysis of the effects of metals on the accuracy of DNA synthesis at three different levels of cellular organization.

At the Level of DNA Polymerases

Current evidence suggests that metal ions can alter the accuracy of catalysis by DNA polymerases from a variety of cells. The fact that infidelity of DNA synthesis correlates with mutagenic or carcinogenic properties of these metals may be only circumstantial when considering the multiple effects of metals in cells. The mechanism by which metals alter the fidelity of DNA synthesis has not been detailed. Careful physical and biochemical measurements are required on the binding of metals to DNA templates and polymerases before mechanistic details can evolve. Of interest is the possibility that particular metals interact preferentially with different nucleotides on DNA templates. An analysis of changes in sequence using natural DNA templates, such as ϕX174 DNA copied in the presence of different metal ions, should provide more definitive results concerning specificity of metal-nucleotide interactions.

At the Level of DNA Replicating Complexes

The most faithful DNA polymerase copies polynucleotide templates *in vitro* with an error rate approximating 10^{-5}. This rate is considerably greater than the spontaneous mutation rates per nucleotide synthesized observed in cells (10^{-8}-10^{-11}). Clearly, other proteins in cells function in accuracy of DNA synthesis. From studies with *E. coli* DNA polymerase I and T_4 DNA polymerase, it is apparent that DNA replication proceeds by the concerted action of multiple proteins (*3*). Further studies are required to determine the effect of each of these proteins on the replication process. With the establishment of enzyme/protein complexes capable of replicating DNA faithfully *in vitro*, it will be necessary to study the effects of metals on these complexes. An analysis of the effects of metals on the accuracy of catalysis by DNA replicating proteins is perhaps somewhat closer to the complexity involved in chromosomal replication in human cells.

At the Cellular Level

The main problem in studying detrimental effects of metals at the cellular level is that metals are required for many cellular processes. Nevertheless, a number of studies on cells in tissue culture demonstrate the mutagenic potential of metals. Thus, a study of the effects of metals on the fidelity of DNA synthesis *in vivo* is important since replication of DNA is required for the establishment of mutational alterations. It would be desirable to extend these studies using various eukaryotic cells that are defective in different pathways of DNA repair, since these cells would be more likely to exhibit metal-induced mutational alterations. There is also a large gap in knowledge of metal content and localization in cells. An analysis of metal content of eukaryotic cells, particularly in the nucleus and chromosomes, is required to better define the relationship between studies on fidelity *in vitro* and mutation rates in cells.

REFERENCES

1. Sunderman, F. W., Jr. Carcinogenic effects of metals. Fed. Proc. 37: 40 (1978).
2. Sirover, M. A., and Loeb, L. A. Infidelity of DNA synthesis *in vitro*: Screening for potential metal mutagens or carcinogens. Science 194: 1434 (1976).
3. Kornberg, A. DNA Synthesis, W. H. Freeman and Co., San Francisco, 1974.
4. Loeb, L. A. Eucaryotic DNA Polymerases. In: The Enzymes, Vol. X, P. D. Boyer, Ed., Academic Press, New York, 1974, pp. 173-209.
5. Slater, J. P., Mildvan, A. S., and Loeb, L. A. Zinc in DNA polymerases. Biochem. Biophys. Res. Commun. 44: 37 (1971).
6. Springgate, C. F., Mildvan, A. S., Abramson, R., Engle, J. L., and Loeb, L. A. *Escherichia coli* deoxyribonucleic acid polymerase I, a zinc metalloenzyme: Nuclear quadrupolar relaxation studies of the role of bound zinc. J. Biol. Chem. 248: 5987 (1973).
7. Sirover, M. A., Dube, D. K., and Loeb, L. A. On the fidelity of DNA synthesis. VIII. Metal activation of *E. coli* DNA polymerase I. J. Biol. Chem. 254: 107 (1979).
8. Sloan, D. L., Loeb, L. A., Mildvan, A. S., and Feldman, R. J. Conformation of deoxynucleoside triphosphate substrates on DNA polymerase I from *E. coli* as determined by nuclear

magnetic relaxation. J. Biol. Chem. 250: 8913 (1975).
9. Mildvan, A. S. and Loeb, L. A. The role of metal ions in the mechanisms of DNA and RNA polymerases. CRC Crit. Rev. Biochem. 61: 219 (1979).
10. Zakour, R. A., Loeb, L. A., Kunkel, T. A., and Koplitz, R. M. Metals, DNA polymerization and genetic miscoding. In: Trace Metals in Health and Disease, N. Kharasch, Ed., Raven Press, New York, 1979, pp. 135-153.
11. Drake, J. W. Comparative rates of spontaneous mutation. Nature 221: 1132 (1969).
12. Loeb, L. A., Weymouth, L. A., Kunkel, T. A., Gopinathan, K. P., Beckman, R. A., and Dube, D. K. On the fidelity of DNA replication. Cold Spring Harbor Symp. Quant. Biol. 43: 921 (1979).
13. Mildvan, A. S. Mechanism of enzyme action. Ann. Rev. Biochem. 43: 357 (1974).
14. Agarwal, S. S., Dube, D. K., and Loeb, L. A. On the fidelity of DNA replication. VII. Accuracy of *E. coli* DNA polymerase I. J. Biol. Chem. 254: 101 (1979).
15. Battula, N., and Loeb, L. A. The infidelity of avian myeloblastosis virus deoxyribonucleic acid polymerase in polynucleotide replication. J. Biol. Chem. 249: 4086 (1974).
16. Wang, T. S.-F., Sedwick, W. D., and Korn, D. Nuclear deoxyribonucleic acid polymerase. Purification and properties of the homogeneous enzyme from human KB cells. J. Biol. Chem. 249: 841 (1974).
17. Seal, G., Shearman, C. W., and Loeb, L. A. On the fidelity of DNA replication: Studies with human placenta DNA polymerases. J. Biol. Chem. 254: 5229 (1979).
18. Sirover, M. A., and Loeb, L. A. On the fidelity of DNA replication. Effect of metals on synthesis with avian myelobastosis DNA polymerase. J. Biol. Chem. 252: 3605 (1977).
19. Hall, Z. W., and Lehman, I. R. An *in vitro* transversion by a mutationally altered T_4-induced DNA polymerase. J. Mol. Biol. 36: 321 (1968).
20. Linn, S., Kairis, M., and Holliday, R. Decreased fidelity of DNA polymerase activity isolated from aging human fibroblasts. Proc. Natl. Acad. Sci. (U.S.) 73: 2818 (1976).
21. Dube, D. K., and Loeb, L. A. Manganese as a mutagenic agent during *in vitro* DNA synthesis. Biochem. Biophys. Res. Commun. 67: 1041 (1975).
22. Miyaki, M., Murata, I., Osabe, M., and Ono, T. Effect of metal cations on misincorporation by *E. coli* DNA polymerases. Biochem. Biophys. Res. Commun. 77: 854 (1977).
23. Luke, M. Z., Hamilton, L., and Hollocher, T. C. Beryllium-induced misincorporation by a DNA polymerase. Biochem. Biophys. Res. Commun. 62: 497 (1975).
24. Sirover, M. A., and Loeb, L. A. Metal-induced infidelity during DNA synthesis. Proc. Natl. Acad. Sci. (U.S.) 73: 2331 (1976).
25. Radman, M., Vallani, G., Boiteux, S., Defais, M., Caillet-Fauquet, P., and Spadari, S. On the mechanism and genetic control of mutagenesis due to carcinogenic mutagens. In: Origins of Human Cancer, Book B: Mechanisms of Carcinogenesis, Vol. 4, J. D. Watson and H. Hiatt, Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1977, pp. 903-922.
26. Tkeshelashvili, L. K., Shearman, C. W., Koplitz, R. M., Zakour, R. A., and Loeb, L. A. Fidelity of DNA synthesis: Effects of As, Se, and Cr. Proc. Am. Assoc. Cancer Res., 20: 263 (1979).
27. Schrauzer, G. N. Trace elements, nutrition and cancer: perspectives of prevention. In: Inorganic and Nutritional Aspects of Cancer (Advances in Experimental Medicine and Biology, Vol. 91), G. N. Schrauzer, Ed., Plenum Press, New York, 1978, pp. 323-344.
28. Hoffman, D. J., and Niyogi, S. K. Metal mutagens and carcinogens affect RNA synthesis rates in a distinct manner. Science 198: 513 (1977).
29. Weymouth, L. A. and Loeb, L. A. Mutagenesis during *in vitro* DNA synthesis. Proc. Natl. Acad. Sci. (U.S.) 75: 1924 (1978).
30. Gopinathan, K. P., Weymouth, L. A., Kunkel, T. A., and Loeb, L. A. On the fidelity of DNA replication: Mutagenesis *in vitro* by DNA polymerases from an RNA tumor virus. Nature 278: 857 (1979).
31. Kunkel, T. A., and Loeb, L. A. On the fidelity of DNA replication: Effect of divalent metal ion activators and deoxyribonucleoside triphosphate pools on *in vitro* mutagenesis. J. Biol. Chem. 254: 5718 (1979).
32. Tkeshelashvili, L. K., Shearman, C. W., Zakour, R. A., Koplitz, R. M., and Loeb, L. A. Effects of arsenic, selenium, and chromium on the fidelity of DNA synthesis. Cancer Res. 40: 2455 (1980).
33. Battula, N., and Loeb, L. A. On the fidelity of DNA replication. II. Characterization of polynucleotides with errors in base-pairing synthesized by avian myeloblastosis deoxyribonucleic acid polymerase. J. Biol. Chem. 250: 4405 (1975).
34. Chang, L. M. S. Low molecular weight deoxyribonucleic acid polymerase from calf thymus chromatin. J. Biol. Chem. 248: 6983 (1973).
35. Slater, J. P., Tamir, I., Loeb, L. A., and Mildvan, A. S. The mechanism of *E. coli* deoxyribonucleic acid polymerase I: magnetic resonance and kinetic studies of the role of metals. J. Biol. Chem. 247: 6784 (1972).
36. Eichhorn, G. L., and Shin, Y. A. Interaction of metal ions with polynucleotides and related compounds. XII. The relative effect of various metal ions on DNA helicity J. Am. Chem. Soc. 90: 7323 (1968).
37. Murray, M. J., and Flessel, C. P. Metal-polynucleotide interactions. A comparison of carcinogenic and non-carcinogenic metals *in vitro*. Biochim. Biophys. Acta 425: 256 (1976).